

REPORT ON MICROANALYTICAL DETERMINATION OF NITROGEN BY THE DUMAS METHOD

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The Dumas method for determining nitrogen was studied in 1948 (5), 1952 (1), and again this year. The first study showed only that the combustion temperature should be above 650°C. to prevent low and erratic results. Last year each collaborator again used the method normally employed in his laboratory except that temperatures above 650°C. were specified. The results were analyzed statistically to obtain indications as to which variables in the procedures were preferred because they produced better accuracy or precision.

A procedure was written to include those variables indicated to be best by last year's study. This tentative procedure, and samples of the same two materials analyzed last year, were sent to collaborators with the request that they follow the revised procedure as closely as possible. If modification of the procedure by the collaborator was necessary, the changes made were to be called to the attention of the Referee when the results were submitted.

Since the tentative method permitted the analyst a number of choices in the apparatus to be used, the card supplied for reporting results also contained a form to be checked to provide information concerning the apparatus used for each set of results.

The 1952 study indicated that the method of Shelberg (2), or that of Zimmermann (6) which is similar to Shelberg's, might be superior to the conventional micro Dumas method. Because there was not sufficient data available from the 1952 study to evaluate these newer methods properly, those collaborators now using the Shelberg or Zimmermann methods were asked to analyze the samples this year by either of these procedures rather than by the tentative Dumas method.

The micro Dumas procedure submitted to the collaborators for the 1953 study was as follows:

TENTATIVE DUMAS NITROGEN PROCEDURE

REAGENTS

- (a) *Potassium hydroxide soln.*—Dissolve 50 g KOH in 50 ml H₂O.
- (b) *Mercury.*—Previously used or slightly dirty mercury is preferred.
- (c) *Copper oxide; Coarse.*—Wire form, about 1 mm in diam. and 2–4 mm long.
Fine.—CuO wire ground to pass 40 mesh but not 100 mesh sieve. Pre-ignite both at 700–800°C. for 30 min. in nickel or steel crucible and store in glass bottles having 1–2", 7 mm O.D. glass pour-out tubing.

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- (d) *Copper (metal)*.—Clean, degreased copper turnings or wool.
- (e) *Dry ice*.—Solid CO₂. Must give micro bubbles ca 0.2 mm diam. in nitrometer.
- (f) *Asbestos (fiber)*.—Acid wash and pre-ignite to 800°C.

APPARATUS

- (a) *Dewar flask*.—1 liter, for carbon dioxide generator, fitted with 2-hole rubber stopper contg 2 gas delivery tubes, one with stopcock and ball joint leading to app., and other to bottom of cylinder contg 15–18" of H₂O or with one hole stopper containing standard mercury valve (7).
- (b) *Combustion tube*.¹—Quartz or Vycor, 520–530 mm long, 11 mm O. D., 8 mm I.D., with 7/15 S.T. inner joint on tip.
- (c) *Needle valve*.²—Standard (7), stainless steel with 7/15 S.T. glass outer joint cemented to tip with Krönig or deKhotinsky cement and with 12/5 glass ball joint cemented to nitrometer end.
- (d) *Nitrometer*.—Either Stehr or Pregl type (7), 1.5 ml graduated in hundredths, calibrated in intervals of at least 0.2 ml and preferably 0.1 ml and with magnifying lens attachment.
- (e) *Furnaces; Sample burner*.—Electric,^{3,4} 13–14 mm I.D., by 3–4" long, operated at a temp. of 775–800°C. *Long furnace*.—Electric, 13–14 mm. I.D. 8" long, operated at temp. of 750–775°C.

PREPARATION OF APPARATUS

Dewar flask.—Break into pieces $\frac{1}{2}$ " or smaller sufficient dry ice to fill flask, place in flask, insert stopper containing tubes, open stopcock on delivery tube, place pressure tube in cylinder of water, and allow to stand 1 hr for removal of trapped air. Close and open stopcock occasionally during hour. (One filling should supply CO₂ for one week.)

Combustion tube, permanent filling.—Place enough acid-washed, pre-ignited asbestos fibers in clean tube to form a 3–4 mm loose plug in end of tube. Use a glass rod to push asbestos in place. Holding the tube in vertical position, introduce in succession 10–12 cm of pre-ignited coarse CuO (tap tube to settle CuO), 2–4 mm loose asbestos plug, 3–4 cm Cu turnings or wool, 2–4 mm loose asbestos plug, 5–6 cm coarse CuO (tap tube to settle CuO), 2–4 mm loose asbestos plug, and 3–4 cm Cu turnings or wool; cap with 4–6 mm loose asbestos plug. Place tube in long furnace so that tip and ca 5 cm of CuO filling protrudes. Connect CO₂ source by placing one-hole rubber stopper in tube and moistening and inserting tapered CO₂ delivery tube. Sweep 10 min. with CO₂, bring long furnace to temp. with slow stream of CO₂ through tube and after 1 hr make simulated sample burning. Turn off burner and long furnace and allow tube to cool with rapid CO₂ flow.

Nitrometer.—Fill nitrometer with mercury to midway between capillary side arm opening and opening of tubulation for connecting leveling bulb. Place few mg Hg₂Cl₂ on mercury to prevent sticking of gas bubbles. Fill nitrometer leveling bulb with 50% KOH. Connect needle valve to nitrometer side arm through ball and socket joint held together by pressure clamp.

Control analysis.—Make control analysis by the following procedure using standard pure compound and calculate blank correction. Repeat until calculated blanks check to ± 0.003 ml.

¹ Standard tube (7) without joint may be used.

² Precision stopcock and rubber tubing connections may be used.

³ Mechanically operated sample burner preferred.

⁴ A gas sample burner may be used but specified temperature must be obtained.

PROCEDURE

Weigh in micro porcelain boat⁵ sufficient sample to give 0.4–0.8 ml nitrogen. Use micro balance if sample weight is less than 10 mg, otherwise semimicro balance may be used. Disconnect cooled combustion tube, remove from furnace and introduce temporary filling of 7–8 cm of coarse CuO, 1–2 cm fine CuO, boat containing sample, 4–5 cm fine CuO, and 2–3 cm coarse CuO.⁶ Holding tube at angle, rotate and tap to mix sample and CuO. Replace tube in combustion furnaces, connect CO₂ supply, and flush tube with rapid CO₂ stream 3–5 min. Turn on long furnace, or if split type furnace is used pull furnace into position over combustion tube. Connect needle valve–nitrometer assembly (with valve open) by placing small amount of Krönig cement on 7/15 inner joint of combustion tube, warming joint and cement with small flame, and connecting 7/15 outer joint of needle valve. Have leveling bulb in lower position and nitrometer cock open during this operation. When joint has cooled, close valve, raise leveling bulb until KOH level is in bulb above stopcock, close stopcock, and place bulb in lower position. Open valve so that CO₂ flow is 3–5 bubbles/sec. and continue sweeping until micro bubbles (diam. 0.2 mm, or equal to width of calibration lines) are obtained. Reduce CO₂ flow to 1–2 bubbles/sec. when testing for micro bubbles. Displace any N in nitrometer, close stopcock on delivery tube, open needle valve, place sample burner 4" from long furnace, bring burner to temperature, 775–800°C., and move burner over sample at rate of 0.5 cm/min. When gas flow into nitrometer slows or stops, close needle valve, open delivery stopcock, then open needle valve to allow flow of 2 bubbles/sec. Turn off sample burner 3–5 min. after it reaches the long furnace. Continue sweeping at 2 bubbles/sec. until bubble size approaches micro, turn off or remove long furnace, and speed up flow to 3–5 bubbles/sec. When micro bubbles are obtained (check with flow of 1–2 bubbles/sec.), force any KOH that may have leaked through stopcock back into cup by carefully opening stopcock with leveling bulb raised above stopcock, hang bulb in upper position, and let stand 10 min. Gently heat combustion tube joint, disconnect from needle valve, remove tube from furnace when cool, remove temporary filling, and prepare for next sample. Adjust nitrometer leveling bulb so that KOH in bulb and tube are level and read volume of nitrogen using magnifying lens attached to nitrometer. Determine and record temperature of air adjacent to nitrometer, and barometric pressure in room. Calculate % N in sample as follows:

$$\text{Per cent N} = V_c \times \frac{P}{273 + t} \times \frac{44.90^7}{\text{Sample wt (mg)}}$$

$$V_c = (V \pm \text{calib. corr.}) - (V \times 0.011) - (\text{blank correction.}^8)$$

Blank correction.—Analyze standard pure compound by above procedure and calculate correction as follows:

$$V_1 = \% \text{ N} \times \frac{273 + t}{P} \times \frac{\text{Sample wt (mg)}}{44.90}$$

$$V_2 = (V \pm \text{calib. corr.}) - (V \times 0.011)$$

$$V_2 - V_1 = \text{blank correction}$$

V, P, and t are observed values in control analysis. % N is theoretical value for standard compound.

⁵ If sample is liquid, weigh in capillary containing KClO, plug in closed end.

⁶ Amounts of coarse and fine CuO used should not vary markedly because they affect the blank correction.

⁷ $44.90 = \frac{273}{760} \times \frac{28,016}{22,414} \times 100.$

⁸ From control analysis.

RESULTS

Twenty-one collaborators used the 1953 tentative method either as described or with only slight modification. They reported 103 values for sample 1, nicotinic acid. Twenty collaborators also analyzed sample 2, acetone-2,4-dinitrophenyl hydrazone, and reported 94 values for this material.

Each collaborator was asked to report all the data he obtained unless some known error was made in a determination and all values reported have been used to obtain the statistical data in this report.

Table 1 shows the tabulated results for both samples. In this table, n is the number of values reported, \bar{x} the average, s the standard deviation and $s_{\bar{x}}$ the standard deviation of the \bar{x} values.

TABLE 1.—*Summary of results obtained by tentative Dumas method*

COLLABORATOR NO.	NICOTINIC ACID (11.38% N)			ACETONE-2,4-DINITROPHENYL HYDRAZONE (23.52% N)		
	n	\bar{x}	s	n	\bar{x}	s
0	3	11.38	0.20	3	23.40	0.18
2	4	11.55	0.17	4	23.79	0.18
8	5	11.33	0.05	4	23.50	0.12
14	8	11.39	0.11	8	23.55	0.08
15	8	11.03	0.07	8	23.44	0.07
23	5	11.28	0.46	6	23.32	0.33
30	6	11.39	0.08	6	23.44	0.03
31	4	11.40	0.10	4	23.41	0.10
35	6	11.35	0.11	4	23.92	0.12
39	8	11.52	0.10			
44	5	11.06	0.09	5	23.61	0.27
45	4	11.31	0.07	4	23.46	0.13
59	3	11.45	0.07	3	23.40	0.06
71	6	11.68	0.23	6	23.81	0.14
72	4	11.40	0.17	4	23.50	0.19
74	4	11.38	0.07	4	23.90	0.13
75	4	11.17	0.04	4	22.84	0.05
76	8	11.49	0.28	8	23.34	0.10
77	4	11.43	0.07	4	23.47	0.07
79	5	11.33	0.03	5	23.54	0.05
80	5	11.40	0.06	4	23.21	0.14
Total No. 103		21		94	20	
Over-all Mean		11.37	0.13		23.45	0.13
$s_{\bar{x}}$		0.15			0.31	

The over-all means of 11.37 and 23.45 per cent N are only 0.01 and 0.07 per cent lower than the theoretical values, respectively. Neither of these deviations is significant by the t test (3). The average of the standard

deviations is 0.13 for both samples, a reasonably low value. Contrasted with this are the $s_{\bar{x}}$ values of 0.15 and 0.31 for samples 1 and 2, respectively. The value of 0.15 is acceptable but the poor precision between analysts shown by the $s_{\bar{x}}$ of 0.31 for acetone-2,4-dinitrophenyl hydrazone indicates a need for refinement of the tentative method tested or the search for a new and better method.

Each analyst supplied the following information about the procedures used when he reported his results.

- (1) Whether a needle valve or stopcock was used to control the gas flow.
- (2) Whether ground glass joint or rubber connections were used.
- (3) Whether the sample burner was mechanically or manually operated.
- (4) Whether the sample burner was electric or gas fired.

The effect of these variables permitted in the 1953 procedure was examined statistically in the same manner used to evaluate the variables in the 1952 study. The data were divided into groups according to the variable used and F and t tests were applied to each of the four pairs of groups. None of the variables could be shown to be consistently more precise or accurate than its alternate; that is, in no case did the F or t values for both samples exceed the critical F and t values.

Those collaborators who normally use the Shelberg or the Zimmermann procedure were asked to analyze the two samples by either of these, rather than by the tentative method. Table 2 shows the data obtained this year by the Shelberg and Zimmermann procedures and the data obtained last year for the same two materials by these same two procedures.

The over-all mean and standard deviation of the means were calculated for both the data obtained this year and for the combined 1952-1953 data. Even though the combined data include two sets of values by three laboratories, the fact that the sets were obtained one year apart was considered to allow for sufficient variation to treat the two sets as if they had come from different laboratories. This was done to increase the number of values so that a more reliable comparison could be made between these two methods and the tentative method.

The comparison in Table 2 of data obtained by the Shelberg and the Zimmermann methods shows that the Zimmermann procedure produced the more precise interlaboratory results for sample 2, while the results for sample 1 were not significantly different. This is shown by comparing the calculated F 's with the critical $F_{.05}$ value; only for sample 2 does the calculated value exceed the critical value.

In addition to using the tentative method, collaborator 14 analyzed the two samples by the Dumas procedure described by Steyermark (4) with the following results: for sample 1, $n=9$, $\bar{x}=11.33$, $s=0.09$; for sample 2, $n=8$, $\bar{x}=23.37$, $s=0.10$. This collaborator's \bar{x} values by the tentative method were higher and closer to the theoretical value, but his precision by the two methods was similar (see Table 1).

TABLE 2.—Summary of results obtained by Shelberg and Zimmermann methods

COLLAB. NO. ^a	1953			COLLAB. NO. ^a	1952		
	n	\bar{x}	s		n	\bar{x}	s
Nicotinic acid (11.38% N)							
22s	4	11.36	0.07				
27z	5	11.50	0.12				
29z	4	11.52	0.05	29z	4	11.36	0.16
37s	4	11.37	0.14	37s	6	11.35	0.15
63z	4	11.40	0.07	63z	4	11.44	0.05
65s	4	11.63	0.04	67s	4	11.39	0.09
78s	4	11.34	0.04				
1953				1952-1953			
Total No.	29	7			47	11	
Over-all Mean		11.45	(0.08)			11.42	(0.09)
$s_{\bar{x}}$		0.11				0.09	
	n	\bar{x}	s	F	$F_{0.05}$		
Shelberg	6	11.41	0.11	2.47	6.26		
Zimmermann	5	11.44	0.07				
Acetone-2,4-Dinitrophenyl Hydrazone (23.52% N)							
22s	5	23.31	0.13				
27z	5	23.57	0.12				
29z	4	23.64	0.09	29z	3	23.41	0.11
37s	4	23.71	0.16	37s	7	23.63	0.10
63z	4	23.55	0.19	63z	6	23.45	0.09
65s	7	23.62	0.16	67s	4	23.34	0.12
78s	10	23.04	0.30				
1953				1952-1953			
Total No.	39	7			59	11	
Over-all Mean		23.49	(0.16)			23.48	(0.14)
$s_{\bar{x}}$		0.24				0.19	
	n	\bar{x}	s	F	$F_{0.05}$		
Shelberg	6	23.44	0.26	8.34	6.26		
Zimmermann	5	23.52	0.09				

^a The letters s and z after the collaborator number refer to the Shelberg and Zimmermann procedures, respectively.

Collaborator 49 was not able to obtain satisfactory micro bubbles using the high temperatures specified in the tentative method.

Collaborator 9 used his own procedure, which differed from the 1953 tentative method only in the method of correcting the observed volume of nitrogen. He determined a blank correction by burning dextrose, then used the data from different weight samples of a standard material to construct a calibration or correction curve. This was done by plotting the difference between the theoretical volume and the blank corrected, observed volume (STP) against the blank corrected, observed volume. His results were: for sample 1, $n=6$, $\bar{x}=11.39$, $s=0.04$; for sample 2, $n=4$, $\bar{x}=23.45$, $s=0.09$. Whether these values are better than the average values by the tentative method because of the method of obtaining the corrected volume of gas, because of slight changes in procedure, or for some other reason cannot be ascertained from the data available.

The results obtained during the last two years by the Dumas method are summarized in Table 3. In this table, n is the number of analyst's means or \bar{x} 's, \bar{x} is the over-all mean of \bar{x} 's, $s_{\bar{x}}$ the standard deviation of the \bar{x} 's, F the calculated F value, and $F_{.05}$ the critical value from the 5 per cent table. Although $F_{.05}$ is shown, calculated F 's which exceed this critical value show differences significant at only the 10 per cent level because the larger variance, $(s_{\bar{x}})^2$, is arbitrarily placed in the numerator of the equation:

$$F = \frac{(s_{\bar{x}})_a^2}{(s_{\bar{x}})_b^2}.$$

TABLE 3.—Summary of data obtained in 1952 and 1953

METHOD	YEAR	n	\bar{x}	$s_{\bar{x}}$	F	$F_{.05}$
Nicotinic Acid						
Shelberg-Zimmermann	1952-1953	11	11.42	0.09	2.78	2.77
Tentative	1953	21	11.37	0.15		
Collaborator's ^a	1952	23	11.40	0.10	2.25	2.07
Acetone-2,4-Dinitrophenyl Hydrazone						
Shelberg-Zimmermann	1952-1953	11	23.48	0.19	2.66	2.78
Tentative.....	1953	20	23.45	0.31		
Collaborator's ^a	1952	22	23.47	0.17	3.32	2.10

^a Data obtained when each collaborator used the Dumas method normally employed in his own laboratory.

The interlaboratory precision using the tentative method tested this year was significantly lower than with the Shelberg-Zimmermann method and lower than that obtained when the collaborators used their own procedures. The differences were significant at the 10 per cent level for three of the four comparisons. Only for the comparison of the Shelberg-Zimmermann *vs* the tentative method for sample 2 was the calculated F less than the critical value and even here the difference between the two F values was slight. Student's t test (3) was applied to these same data to determine if there were any significant differences between means. None were found so the data in hand do not indicate any of the methods to be more accurate than the other two.

SUMMARY

The tentative micro Dumas method tested this year did not give results with as good interlaboratory precision as was obtained when the collaborators used the procedures they normally employed. The interlaboratory precision obtained by the Shelberg and Zimmermann methods was also better than that obtained by the tentative method and equal to that obtained by the more conventional Dumas procedures normally used. The over-all means by all methods agreed well with the theoretical values for the two samples analyzed, nicotinic acid and acetone-2,4-dinitrophenyl hydrazone.

It is recommended* that the 1953 tentative method should be revised before further collaborative work is done and that a rapid method similar to that of Shelberg or Zimmermann should also be tested collaboratively.

LIST OF COLLABORATORS

V. A. Aluise, Hercules Powder Company; C. J. Bain, Picatinny Arsenal; L. M. Brancone, Lederle Laboratories; L. E. Brown, Southern Utilization Research Branch; W. L. Brown, Eli Lilly & Company; B. L. Browning, Institute of Paper Chemistry; A. W. Dearing, Hunter College; T. DeVries, Purdue University; L. Dorfman, Ciba Pharmaceutical Company; K. K. Fleischer, Sterling-Winthrop Research Institute; E. E. Gansel, Ansco; J. Grodsky, Ortho Research Foundation; G. M. Gustin, Celanese Corporation of America; E. W. D. Huffman, Huffman Microanalytical Laboratory; G. A. Jones, E. I. duPont de Nemours & Company; D. F. Ketchum, Eastman Kodak Company; J. A. Kuck, American Cyanamid Company; J. A. Means, Chas. Pfizer & Company, Inc.; C. W. Nash, Rohm and Haas Company; P. B. Olson, Minnesota Mining & Mfg. Company; J. K. Owens, E. I. du Pont de Nemours & Company; P. Rothmund, Charles F. Kettering Foundation; E. T. Scafe, Socony-Vacuum Oil Company; S. A. Shrader, Dow Chemical Company; J. Sorensen, General Electric Company; A. Steyermark, Hoffman-LaRoche, Inc.; S. J. Tassinari, National Dairy Research Laboratory, Inc.; W. H. Throckmorton, Tennessee Eastman Corporation; C. H. Van Etten, Northern Utilization Research Branch; C. L. Ogg, Eastern Utilization Research Branch.

* For report of Subcommittee A and action of this Association, see *This Journal*, 37, 63 (1954).

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